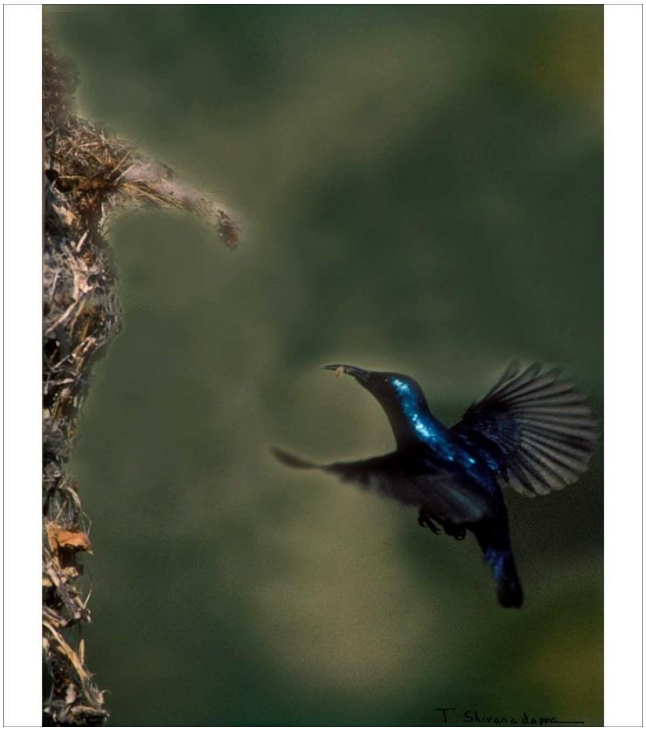

STRUCTURAL AND COMPOSITIONAL ANALYSIS OF SUNBIRD NECTARINIA ASIATICA

THESIS SUBMITTED FOR PARTIAL FULFILLMENT OF THE MASTERS OF SCIENCES DEGREE IN LIFE SCIENCE

BY

GUDRA HEMBRAM

413LS2031

UNDER THE GUIDANCE OF:

DR. MONALISHA MISHRA

ASSSISTANT PROFESSOR

DEPARTMENT OF LIFE SCIENCE

NATIONAL INSTITUTE OF TECHNOLOGY

ROURKELA- 769008

ODISHA

राष्ट्रीय प्रौद्योगिकी संस्थान
NATIONAL INSTITUTE OF TECHNOLOGY
राउरकेला ROURKELA - 769008, ओडिशा ODISHA

RIGHT TO INFORMATION

CERTIFICATE

This is to certify that the thesis entitled "**Structural and compositional analysis of feather Nectarinia asiatica**" which is being submitted by **Mr Gudra Hembram,** Roll No. **413LS2031,** for the award of the degree of Master of Science from National Institute of Technology, Rourkela, is a record of bonafide research work, carried out by him under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

Monalisa Mishra
Dr.Monalisa Mishra
Assistant Professor
Department of Life Science
National Institute of Technology
Rourkela – 769008, Odisha, India

फोन Phone : (0661) 2476773, फैक्स Fax : (0661) 2462022, वेबसाइट Website : www.nitrkl.ac.in

DECLARATION

I do hereby declare that the Project Work entitled ***"Structural and Compositional Analysis of feathers of Sunbird Nectarinia asiatica"***, submitted to the Department of Life Science, National Institute of Technology, Rourkela is a faithful record of bonafide and original research work carried out by me under the guidance and supervision of Dr. Monalisha Mishra, Assistant Professor , Department of Life Science, National Institute of Technology, Rourkela, Odisha.

Date: Gudra Hembram

Place : Rourkela Roll No 413LS 2031

TO MY PARENTS ANDS FRIENDS

ACKNOWLEDGEMENT

First and foremost I bow down before the almighty God who has made everything possible.....
It is a great pleasure and proud privilege to express my deep sense of gratitude and everlasting indebtedness to my research supervisor, **Dr. Monalisha Mishra**, Assistant Professor, Department of Life Science, NIT, Rourkela. I am grateful to him for providing me substantial knowledge, incisive guidance, helpful advices and moral support all the time during my project work. I would like to thank her for patiently scrutinizing the preparation of this project report and making my work a successful one.
I would like to express my sincere thanks to all the faculty members and staffs of Department of Life Science for their constant support and encouragement throughout my M.sc years.
I am highly obliged to my mentor Mr. **Debabrat Sabat** for his patience, immense support and untiring supervision in the experimental works, interactive discussions and motivational encouragements in successfully carrying out this piece of work.
I am extremely thankful to Ms.Sibani Moharana , Ms. Suchismita Sethy, for their constant support,and help during the six months of project work.
My special thanks to **Zoheb Abai**, my room mate for his ever-present support, encouragement and helpful suggestions and discussing the different aspect of nature of light ,several philosophical scientific approaches in experimentation and instilling every other morning the scientific temperament and ideas during my project work.

Last but not the least, I would not have been able to complete this project without the love and support of my parents whose immense faith in my abilities helped me to overcome many obstacles and march ahead during all the difficult times. I would like to dedicate this project to my beloved parents and sisters.

Gudra Hembram

CONTENTS

LIST OF FIGURES

LIST OF GRAPHS

ABSTRACT

The plumage of many birds is highly attractive, especially when thefeathers are patterned in strongly contrasting colours. Orange colours are generally caused by pigments that selectively absorb short-wavelength light. When these pigments are embedded in a diffusive medium, only the long-wavelength part of incident broadband light is reflected and scattered. In contrast, blue or green animal coloration is virtually always due to periodic structures that reflect and scatter incident light of a restricted short-wavelength range(Srinivasarao, 1999; Vukusic and Sambles, 2003; Kinoshita and Yoshioka, 2005; Prum, 2006). Pigmentary and structural coloration are often found simultaneously, not only in birds but also in many other animals, for example butterflies, beetles and lizards (Kinoshita,2008). Birds possess various pigment classes, for instance carotenoids, pterins, porphyrins, psittacofulvins and melanins (McGraw, 2006; Hill and McGraw, 2006), and various mechanisms of structural colouration, namely thin films, multilayers, photonic crystals, keratin spongy nanostructures and nanofibres (e.g. Durrer, 1977; Shawkey et al., 2003;Shawkey et al., 2006; Yoshioka et al., 2007; Doucet and Meadows, 2009; Prum et al., 2009; Stavenga et al., 2010; D'Alba et al., 2011). The predominant location of colouration is the feathers, often either the barbs or the barbules. Structural coloration of avian skin have been long hypothesized to be produced by incoherent(Rayleigh /Tyndall)scattering. Avian plumage color have emerged recently as model system for investigating the type of information that can be signaled by showy sexual display in birds.The non pigmentary colors of the tissues of living organism are produced by physical interaction of light with nanostructures in the tissues. The brilliant iridescent color appearances of many avian feathers are produced by complex optical phenomena. They principally arise from coherent light scattering from self-assembled quasi-ordered structures that have a spatially periodic variation in refractive index. Iridescent structural colors in biology exhibit sophisticated spatially-varying reflectance properties that depend on both the illumination and viewing angles. The classification of such spectral and spatial information in iridescent structurally colored surfaces is important to elucidate the functional role of irregularity and to improve understanding of color pattern formation at different length scales

KEYWORDS: Structural colouration, nanostructures, iridescent,SEM, UV , plumage,

INTRODUCTION

Sexual Dimorphism(SD) is the phenotypic difference between male and female of the same species. The main prototypical example is difference in characteristic of reproductive organs.The body size ,physical strength and morphology are the examples of secondary sexual characteristic. It is a pattern that is seen throughout the animal kingdom and is exhibited in a myriad of ways. Sexual dimorphism is also exhibited in ornamentation, such as the horns of dung beetles (Watsons NL, Simmons LW,2010),the antlers of cervids (Geist V,Bayer M,2009) and the tail of peacocks (Loyau A Saint Jalme M,Cagniant C,Sorci G,2005). Many species also exhibit sexual differences in foraging behavior such as the Russian agamid lizard (Ananjeba NB ,Tsellarius AY,1986), and parental behavior and territoriality can be dimorphic in species such as hummingbirds (Stiles FG, 1971,Armstrong DP ,1987).Another common pattern is that of sexual size dimorphism, such as is observed in snakes
(Shine R,1978) and monk seals (Ralls K).

There are many mechanisms that drive the evolution of SD, the most accepted mechanism being sexual selection (Hedrick AV,Temeles EJ,1989,Abouheif E ,Fairbairn DJ,1997,Andersson M,1994), which enhances fitness of each sex exclusively in relation to reproduction (Darwin CR, 1871,Jones AD, Ratterman NL,2009). This states that SD evolves in a direction such that each sex maximizes reproductive success in two ways: by becoming more attractive to the other sex (inter-sexual dimorphism) or by enhancing the ability to defeat same-sex rivals (intra-sexual dimorphism), in both cases such that each sex increases the chances to mate
and pass genes on to the next generation. Many researchers have argued that competition for mates is at the very heart of sexual selection because these rivalries greatly influence mating and fertilization success. Indeed, competition for mates has been shown to be the major factor impacting SD in several taxa (Bean D , Cook JM,2001). However the complexity of SD cannot be explained by a single mechanism.
Mate choice is an important proximate mechanism of sexual selection. Often the sex with the higher reproductive investment is the ‘choosy’ sex. Patterns then emerge, such as those consistent with the ‘sexy son’ hypothesis (Weatherhead PJ, Robertson RJ,1979), where females prefer mates with phenotypes signifying fitness. The females prefer males that are phenotypically ‘sexy’ to ensure that the genes of their offspring will produce males that will have the most breeding success,

propagating her genes successfully (Jones AD, Ratterman NL,2009,Hunt J,Breuker CJ,Sadowski JA, Moore AJ). Taken further, sometimes females prefer males that exhibit very extreme phenotypes within a population. Over evolutionary time these traits become increasingly exaggerated despite the potential fitness costs to the males themselves, termed Fisherian runaway sexual selection (Weatherhead PJ, Robertson RJ,1979). Examples include the tails of male peacocks, plumage in birds of paradise and male insect genitalia (Andersson M 1994,Fisher RA 1915,1930).

Hypotheses continue to be proposed and the explanations for the evolution of SD may not be mutually exclusive but instead, may operate in a synergistic or antagonist fashion to shape these patterns.

Process and pattern of Sexual Dimorphism

Sexual size dimorphism is a frequent phenomenon where the size of males and females of the same species differ driven by one or more of the mechanisms mentioned above. When these processes occur in closely related species, distinct patterns of among species size dimorphism can result, one of which is termed 'Rensch's Rule' (Rensch B,1950). Rensch's rule is a pattern wherein the degree of sexual size dimorphism increases with body size in species where males are the larger sex, and conversely decreases in those species where females are the larger sex

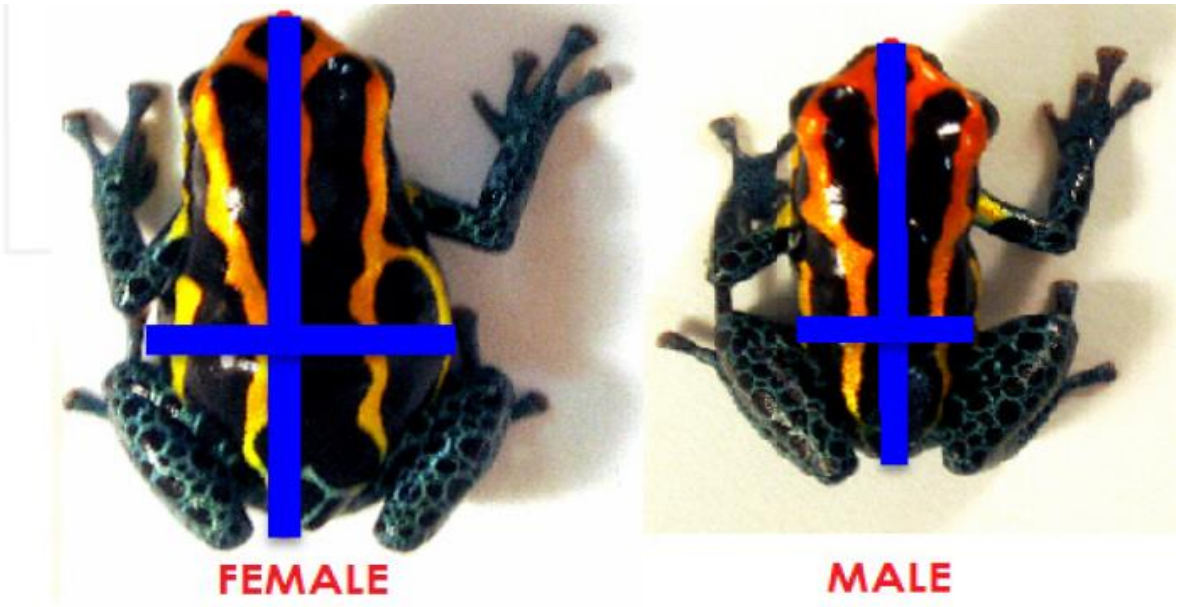

Figure 1:Sexual size Dimorphism in poison dart frog

Several hypotheses have been proposed to explain Rensch's rule. One proposes that the combination of genetic correlations between male and female size with directional sexual selection for larger male size will cause the evolution of larger males relative to female body size (Abouheif E, Fairbairn DJ,997; Fairbairn DJ,Preziosi RF,1994;). Another argues that sexual size dimorphism evolves through intraspecific competition between the sexes when foraging is related to size (Darwin CR, 1871;Shine R 1989). Finally, many researchers have hypothesized that this pattern is due to female fecundity, where the larger female will have bigger eggs and a greater capacity to reproduce successfully (Darwin CR, 1871;William GC, 1966;Hughes AL , Hughes MK ,1986).

Examples of Rensch's rule and support for all three hypotheses abound in nature in organisms as diverse as hummingbirds (Collwell RK, 2000), hummingbird flower mites (Collwell RK, 2000), water striders (Fairbairn DJ, and, Preziosi RF 1994), turtles (Berry JF, 1980), salmon (Young KA ,2005)and shorebirds (Székely. T, Freckleton RPa, Reynolds RJ,2004).

Sexual Dimorphism in Nectarinia Asiatica

The most prototypical characteristic of Sexual Dimorphism is reproductive organs .There is a striking difference in dimorphic characteristic in sunbird. The male bird is generally deep dark in color uniformly ,its tail part being black .On the other hand the female bird is yellowish in color with white color inclined to the undertail region of wing.

Adult male

This small sunbird has a relatively short bill, a dark and short square ended tail with distinctive sexual dimorphism. Less than 10 cm long they have a down-curve bill with brush-tipped tubular tongues that aid in nectar feeding.The general colour of the whole bird is metallic black.The wings are black above edged with bluish,the under surface is brown,without any pale inner edges.The tail feathers are uniformly black.The sides of the neck , and the undertail coverts are dark purplish blue. The chin and throat are also dark purplish blue,the terminal part of many of the feather of the chest are marooned red.The large pectoral tuft tufts are orange and bright yellow.The breast , abdomen flanks and underwing coverts are velvety purple gloss.

Adult female

Females are olive brown above with yellowish underside. The inner web of quills edged with pale buff.The tail is blackish with large white tips to most of the feather.All the underparts are uniformly yellow,this coloured inclined to white on the undertail and wing coverts are on the edge of the wing.The thighs are dull yellow,sides slightly tinged with green.There is a pale supercilium beyond the eye. There is a darkish eye stripe. The throat and breast are yellow becoming pale towards the vent. The outer tail feathers are tipped in white both in the male and female

Feather

Feathers are the most distinctive features of bird. They are extraordinary evolution of invention.They are fundamental to many aspects of birds existence mainly responsible for the aerodynamics,insulation,communication and camouflage.The primary function are to provide protection ,insulation and capacity for flight.Insulation is essential to regulate body temperature.Feather colour is important for communication(mate selection ,territory dominance) and camouflage.Modified wings are important for swimming, sound production(communication),hearing owls,tactile sensations(night hawks),breeding displays.

Feather structure

The central shaft is called as rachis.The broad flat structures present on both sides are called vanes.The base of the feather is called calamus.It anchors feather into follicles.The inferior umbilicus provides access to blood flow during development.The veins grade from hidden

fluffy ,insulating section at base which arise from plumaceous section.The pennaceous section are present on the distal end which are clearly visible.The lateral branches off the rachis are called barbs and primary sturucture.The central shaft of a barb is called as ramus.Each barb is divided into more branches called barbules. Barbules consists of a single cells linked end to end, bearing many barbicels or hooklets.The barb and barbules form an interlocking strong flexible surface and one of the most precisely adapted epidermal structure in the animal kingdom.

The pennaceous feather is further divided into contour ,bristles and flight.The plumaceous are categorized into semiplume filoplume and down.The contour feather are basic vaned feather of body and wing.It includes large flight feathers of wings and tail with asymmetrical vanes.Smaller contour feather cover body aand have symmetrical vanes.The regimes and retrices are a subset of contour feather.It includes flight feathers of the wing ,including primaries ,secondaries and tertiaries and tail feather.Feather vanes are usually asymmetrical. Bristles feathers are contour feathers without vanes.It consist of rachis but lack barb and barbules.They arefound around the eye for protection, and around the mouth for tactile sensivity(insectivorous species).

Semiplumes are intermediate between pennaceous (stiff) and plumaceous(down) feathers which lack a rachis.Semiplumes are distinguished from down feather by having a rachis that is longer than any barb. Semiplumes lie at the edge of contour feather tracts,provides insulation,and serve in courtship display.

Filoplumes are longer hair like feathers that monitors the position of the pennaceous feather on the wind and tail.Sensory corpuscles at the base of each filoplume detect fine movement in feather shaft.Each flight feather may have 8-12 filoplumes.They are most abundant at the base of the wing.

There are plumaceous feather that provide a layer of insulation under the contour feathers.Down feather usually do not have a rachis or it is shorter than the barbs.

The feather is distributed mainly over the eight major tracts namely capital ,ventral, humeral,alar, femoral,crural, caudal and spinal.The capital tract extends over the entire dorsal surface of the head.The ventral tracts cover the ventral neck,breast and abdominal region.Humeral tract is associated with a band of contour feathers that overlie the humerus on the dorsal side of the wing. Includes all the major feathers(primaries,secondaries,alula,tertials)Femoral tract cover the thigh upward to the base of the tail.Crural tract includes lower leg feathers.Cudal tract includes the major flight feathers

of the tail(the retrices).Spinal tract runs down the dorsal midline of the body from the base of the skull to the pygostyle(fused caudal vertebrae)

Bird Colouration

Bird colouration has captured the imagination of naturalist and researchers since Darwin (1871) first publish on sexual selection.Recently new insight on sexual selection(Hamilton and Zuk,1982),advances in understanding avian vision (Cuthill et.al 2000) and measurement of colour (Endler 1990) have brought renewed and vibrant interest to study colouration, both from mechanistic and evolutionary from of view.Bird colouration is divided into two volumes the first which focuses on the perception and measurement of colour, and also the mechanism of colour production and control.The second volume treats the function and evolution of colour often but not exclusively, focusing on sexual selection.

Numerous taxa such as insects, fish, amphibians, reptiles,birds and mammals display preferences for exaggerated colours (Andersson, 1994). Greater colour intensity, large coloured patches or more coloured patches are apparently because of open-ended preferences for even more exaggerated traits (Ryan & Keddy-Hector, 1992).Although there are extensive studies of the functional benefits of such preferences, the origin of the preferences is poorly understood. Some of the other school of thought about the colouration is related to the nutritional habit of bird which are described as follows.

Secondary sexual traits may have evolved within or outside a sexual selection context. Sexual preferences may originate from a foraging context, if food items of such characteristics have become preferred because of their nutritive qualities (Rodd et al., 2002).Such as preference may then also be expressed in a sexual context, and become further exaggerated, if acquisition of mates with such features is associated with a fitness advantage. This scenario predicts colour preferences

being related to the colour of food, and that sexual colour preferences subsequently evolved as a consequence of pre-existing bias. Once strong preferences for sexual colouration have evolved, we could also expect colour preferences to be expressed outside the sexual context in which theyoriginally evolved. That should particularly be the case when there is little or no cost associated with the expression of such preferences. Alternatively, colour preferences in a sexual context are independent of colour of food. Gizzard stones that birds ingest to facilitate mashing up their food may constitute such an example.

The colour of grit reflected innate colour preferences related to plumage colour.There are lot of question arising these hypothesis like.Firstly, to which extent colour of grit is a repeatable feature of a

species.Secondly, whether colour of grit is positively correlated with colour of the body, especially sexually dichromatic body colour (i.e. colour of the plumage and soft parts).

Structural Colouration

Structural colourationis the production of colour by microscopically structured surfaces, sometimes also called schemomeres fine enough to interfere with visible light sometimes in combination with pigments. From a scientific point of view, the explanation of the origin of the colors observed

belongs mainly to the m´etiers of physics and chemistry, but the implications of the presence of coloration in different materials extend to many other disciplines. In particular, coloration as mean of communication plays a crucial role in many areas of biology and the study of species capable of analyzing the complicated color signals. Among such species are certainly humans, and color and coloration play a central role in many situations extending from the scientific, through the practical, to the aesthetic aspects of our lives. Colors allow us to discover and understand physico/chemical phenomena taking place both at microscopic scales invisible to our eyes and at intergalactic distance in our universe; they code, guide, warn, and help us in many aspects of our everyday lives, and they are also capable to stimulate our minds, provoke emotions

Structural coloration was first observed by English scientists Robert Hooke and Isaac Newton, and its principle – wave interference – explained by Thomas Young a century later. Young correctly described iridescence as the result of interference between reflections from two (or more) surfaces of thin films, combined with refraction as light enters and leaves such films. The geometry then determines that at certain angles, the light reflected from both surfaces adds (interferes constructively), while at other angles, the light subtracts. Different colours therefore appear at different angles. Structural coloration is caused by interference effects rather than by pigments. Colours are produced when a material is scored with fine parallel lines, formed of

one or more parallel thin layers, or otherwise composed of microstructures on the scale of the colour's wavelength. Iridescence, as explained by Thomas Young in 1803, is created when extremely thin films reflect part of the light falling on them from their top surfaces. The rest of the light goes through the films, and a further part of it is reflected from their bottom surfaces. The two sets of reflected waves travel back upwards in the same direction. But since the bottom-reflected waves travelled a little further – controlled by the thickness and refractive index of the film, and the angle at which the light fell – the two sets of waves are out of phase. When the waves are one or more whole wavelength apart – in other words at certain specific angles, they add (interfere constructively), giving a strong reflection. At other angles and phase differences, they can subtract, giving weak reflections. The thin film therefore selectively reflects just one wavelength – a pure colour – at any given angle, but other wavelengths – different colours – at different angles. So, as a thin-film structure like a butterfly's wing or bird's feather moves, it seems to change colour.

Structural colouration in birds

Among the most colourful object found in Nature are bird feathers.Their beauty is more obvious and mesmerizing when the bird is in flight as the colour of the pigments present in the wing and feather is accentuated by the phenomenon of refraction and reflection of incident light by the nanokeratinized structural element in the feather (Krishnaswamy NR, Sundaresan CN).

Both chemical pigments and the physical aspects of the wavelike behavior of light are responsible for the coloration of birds. Pigments have the property of absorbing and emitting selective wavelengths of the ambient light. The resulting colors are determined by the molecular structure of the pigments. Such pigments may be synthesized by the birds themselves or acquired by the birds through their

diet. By removing the pigmentary substance from the tissues the colors disappear, verifying that the pigments are the cause of the coloration. A typical example of pigmentary coloration is provided by flamingos, whose recognizable pink color tends to fade out in captivity through a modification of their diet from the one they have in the wild. Likewise, the black or brown colors of the feathers of a crow or a robin are produced by melanin pigments synthesized by the animal, just as in human

black or red hair.

Unlike pigmentary colors (usually yellows, oranges, reds, browns, and blacks) structurally produced colors in avian tissues (often blues and greens) are the result of the physical

interaction of light with optical heterogeneities of the tissues.Incoherent Rayleigh scattering has been erroneously assumed to be responsible for the observed non-pigmentary colors of many birds. Rayleigh (or Tyndall) scattering

occurs when small, light-scattering objects are randomly distributed without a spatial pattern in the path of the light. Small objects will preferentially scatter smaller wavelengths, giving rise to a bluish or violet color. This mechanism is the explanation for the color of the blue sky. According to this conception of biological structural color, small melanin granules present in the feathers or skin of bird tissueswill reflect back short waves, such as violet and blue, but will let pass through longer waves such as red and yellow

Plumage color of both males and females is most commonly derived from one of three distinct mechanisms:carotenoid pigments, melanin pigments, or feather microstructure(Fox and Vevers 1960; Hill and McGraw 2005).Variation in noniridescent structurally based coloration is largely a function of the anatomy the spongy layer of feather barbs (reviewed in Prum 1999)

REVIEW OF LITERATURE

T he colour of feather in birds, its so luminous and attractive what is the reason behind it .Is it the property of birds that illuminates the bird , or does it reside in feather themselves.From peacock blue color to swan white to oriole orange,every bird color is produced by the interaction of just two coloring system-one structural and one chemical. Structural color results from the scattering of reflected light, while chemical color relies on a palette of pigments. Intricately arranged feather layers allow chemistry and structure to interact to produce the colors we see. feathers grow in a symmetrically branching pattern, resembling the leaves of ferns. In each feather, small branches called barbs grow out of a central shaft, and smaller branches called barbules grow out of each barb. Long rows of barbules lie in flat, overlapping rows on adjacent barbs. The geometric arrangement is held in place by hook-like structures on the undersurface of the barbules that lock them together like zippers. When barbs separate, feathers look ruffled and uneven. When birds preen, they zip up the barbules and reattach the hooks, making the feathers lie smooth.

In the cross section of barb under microscope, we can see central core surrounded by a layer of color producing structures and an outer region called cortex. Pigments in either in core or cortex are responsible for the color production of feather, but colors also occurs in the region in feather where cortex is pigment free. In these feathers, the layer between core and cortex – called either the cloudy zone or the spongy layer due to the appearance of a dissected feather to the naked eye – produces colors through convoluted air cavities that act as tiny light-scattering prisms. Sexually Dichromatism in birds is thought to have arisen from a dull monochromatic state through sexual selection favouring conspicuos colours in male(Darwin,1871; Wallace 1899).This view has been challenged in modern times by number of workers round the globe. Dichromatism is an ancestral rather than a derived state and its expression may be caused by selection for duller plumage in one sex. Genetic drift and indirect selection may also played role in creating or monitoring dichromatism(reviewed in Badyaev & Hill 2003).Some birds species are dichromatic in between the ultraviolet (UV) and the visible spectra(Anderson & Amendsen 1997;Keyser & Hill 1999,Mays et al 2004), or in the UV region alone (Hunt et al 1999,Mayer & Kempenacurs 2000;Eaton & Lasyon 2003)

The primary colors are Red ,Green and Blue in short (RBG) are the visible spectrum for the human being with normal vision. There is a major difference between the trichromacy of humans and the tetrachromacy of such birds.Birds are tetrachromatic possessing ultraviolet(UV) sensitive cone cells in the eye as well as red, green and blue one. This allows them to perceive ultraviolet light , which involve in courtship.Birds have light sensing cells deeper in their brains that respond to light without input from eyes or other sensory

neurons.Many birds shows plumage pattern in ultraviolet that are invisible to human eye. Some birds whose sexes appear similar to the naked eye are distinguished by the presence of ultraviolet reflective pattern on their feathers.Male blue tits have an ultraviolet reflection

crown patches which is displayed in courtship by posturing of their nape feather. Ultraviolet light is also used in foraging kestrels have been shown to searching for prey by detecting the UV reflection in urine trail marks left on the ground by rodents.

Structural colors are prevalent in nature and generally produce by the selective scattering and reinforcement of specific bands of wavelength from biophotonics nanostructures with variation in refractive index on the order of visible wavelength of light.The ultraviolet blue(in our case yellow) feather color is caused by coherent scattering of light within the medullary " spongy layer" of feather barbs . The spongy layer lies beneath the keratin cortex and on top of a layer of melanin granules that surround a hollow central vacuole.Irregularly shaped electron-dense regions are present within cortex.The color of non- iridescent UVor UV blue feather is thought to be produced on a function of the size and arrangement of nanostructural element within the medullary "spongy layer" of feather barbs(Gadow,1882;Dyck,1971; Prum et al 1998,1999,; Prum ,Anderson ,Torres 2003) .

Many bird species possess plumage that reflects ultraviolet (UV) wavelength.Some species of parrot (Psittaciformes) have fluorescent plumage which absorbs short wavelength (UV or blue) and re –emits them at longer wavelength, making the plumage literally "glow"(Boles 1990 ,1991;Pearn et al 2001,Arnold et al 2002).Parrot species that posseses both fluorescent and UV reflecting plumage often juxtapose these colors.Birds use both UV reflective plumage and fluorescent plumage as cues in mating choice. (Bennett *et al.* 1996, 1997; Amundsen *et al.* 1997; Andersson & Amundsen 1997;Andersson *et al.* 1998; Johnsen *et al.* 1998; Hunt *et al.*1998, A1999; Pearn *et al.* 2001; Arnold *et al.* 2002). There is still an ongoing debate whether these signals play an important role in sexual communication or they are simply part of general colouration.(Hunt et al 2001) carried out a experimental study which demonstrated that the UV band is not special importance for mate choice in zebra finch *Taenipygia guttata*.Birds are known to have tetrachromatic color vision system that appears to be extremely conservative(Govardovskii 1983;Vorobyev et al 1998;Hart 2001) In the Passeriformes and Psittaciformes, the four cone types are generally classified as 'UVsensitive'(UVS), 'short-wavelength sensitive' (SWS), 'mediumwavelengthsensitive' (MWS) and 'long-wavelength sensitive'(LWS).Structurally based ultraviolet (UV) colouration of plumage can signal male quality and plays a role infemale mate choice in many bird species.Competition between male over limited resource and potential mates occurs in many avian mating system and can together with female mate choice, drive the evolution of male plumage color that have social signaling functions(Andersson 1994).Such colour badges of status allow competitors to assess each other fighting ability from distances, and hence to settle down contest without risking injuries during aggressive interactions(Rowher ,1975,1982;Whitfield 1987).Cost of the production and maintenance of bright plumage colouration could be socially induced,increased predation risk,direct energetic or nutrient limitation, or hormone mediated(Moller ,1987; Folstad &

Karter ,1992;Andersson,1994; Slagsvold et al 1995;Plumage deposition is due to pigment deposition in the feathers(carotenoids and melanin ;Olson and Owens,1998;Jawor and Bristwisch,2003) or to microstructures in the feathered barb(Fox ,1976 ;Prum et al ,2003). These

microstructures can not only produce blue, violet iridescent colours , but also ultraviolet(UV) which is invisible to humans. UV plumage colours can signal several aspects of male quality, such as survival prospects (Sheldon et al., 1999; but see Delhey & Kempenaers, 2006), parasite load (Doucet & Montgomerie,2003), nutritional condition (Keyser & Hill, 1999; McGraw et al., 2002) and territory quality (Keyser & Hill, 2000).Further studies have shown that female prefer male with high UV reflectance than over less UV reflective male(Andersson and Amudsen ,1997;Andersson et al 1998;Hunt et al 1998; Johnsen et al.Male secondary characters that function in female mate choice cues often play an important role in male – male competition and vice versa(Berglund et al ,1996). In the blue tits(*cyanistes caereleus)*in which both sexes have UV reflecting blue crown feathers, territorial males showed high level of aggression towards male taxidermic mount with natural crown UV reflectance.(Alonso – Alvarez et al 2004).

OBJECTIVE

1.To analyze the relative concentration of pigments in producing the numerous iridescent coloration in the feather of Nectarinia asiatica.

2.To analyze the contribution of biophotonic nanostructures to natural color production in the feather of Nectarinia asiatica.

MATERIALS AND METHODS

Collection of feathers

The feathers were collected from the BITS Pilani campus area.Atotal number of 18 feather were taken for different analysis.Three different coloured varieties of feathers were taken from the Nectarinia asiatica species. Basically orange red ,black and yellow feather were chosen for the study.The sample feather are significantly of different sizes the black feather are relatively bigger than the orange and yellow feather.The feathers are mainly from the three regions of the body namely the head region, the breast region and the lesser coverts region.

GEL doc (Dry UV measurement)

The feathers are placed in the GEL doc system (BIORAD) installed in Microbiological lab of NIT Rourkela to detect for the presence of UV in the different parts of the feathers. The imaging procedure is carried out by the GEL doc, and it is closed for few mins to see the presence of UV. The feathers are then removed and the photographed image is obtained from the system.

Scanning Electron Microscope (SEM)

The basic principle of Scanning Electron Microscope is that it produces images of a sample by scanning it with focused beam of electron.The electrons interact with the atoms in the sample producing numerous signals that can be detected which contain information about the sample surface topography and composition. The electron beam is generally scanned in a raster scan which is flat rectangular surface, and the beam position is combined with the with the detected signal to produce an image.The specimen are normally observed in low vacuum ,high vacuum, in dry conditions or at wide variety of cryogenic conditions.

We carried out or experiment with completely dry samples of the feathers.The feathers are fixed with the ethanol to preserve and stabilize their structure.The feathers are coated with the JEOL,JFC-1600(autofine coater).Then the coated samples are then carried and placed at the specimen stage of the SEM model-JSM-6480LV (NIT Rourkela).

Solid UV

Reflectance and absorbance of the main and side tail feather measured using solid UV-Vis spectrophotometer (JASCO V650). After the measurement of reflectance the feather was subjected to extraction of pigments. The pigments (the lipid soluble pigment like psittacofulvin and **non-melanin** pigments) were extracted from sunbird feathers using the method described by **D'Alba et al., 2012**. Briefly, the acidified pyridine was prepared by mixing of 3 drops of concentrated HCl in 50 ml pyridine (Merck). 1 ml of acidified pyridine were added to the test tubes that contains sunbird feathers. This step is immediately followed by incubation of the sample in preheated water bath at 95°C for 4 hours. After completion of appropriate time period all the tubes were kept at room temperature for cooling. The samples were rinsed with 5 ml tert-butylmethylether: hexane (1:1, v/v) and 1 ml distilled water. After this step, the spectral changes in feather reflectance were measured by UV visible spectrophotometer. The absorbance spectrum (from 300 to 700 nm) of extracted yellow pigment was also determined by UV-visible spectrophotometer (Cary Series UV-Vis spectrophotometer). Along UV measurement, we have done XRD of extracted pigment from sunbird feather to detect the presence of copper and zinc metal.

X –Ray Diffraction (XRD)

The feather sample we have taken are detected using the XRD (Department of Physics NIT Rourkela)for the presence of any metal in it.XRD is used to determine the arrangement of atom in a crystal. An X-Ray beam is passed through the crystals, when this happenslight is diffracted and a pattern that is synonymous to the structure of the crystal is formed. This pattern can be mapped onto the electron density map and analyzing this map shows us the exact arrangement of atoms in the crystals.The crystals must be small in size less tham 1 mm.They must be perfect without cracks ,no inclusion such as air bubbles. If the crystals are not perfect then the end image that is formed will have random patterns or have other problems. Different substance crystallizes differently,small molecules crystallizes easily whereas on the other hand proteins or nucleic acids are harder to crystallize. Crystals are mounted in a way so that the sample can be rotated and an X-Ray beam can be passed through the sample.Methods of mounting include using either a capillary or a tube.Both capillary and tubes are mounted on a goniometer.Crystals need to be positioned within ~25 micrometers accuracy of the beam.Once the crystals are correctly mounted, they are exposed to X-Ray Beams.X-Ray Sources include:Synchrotron: gives high resolution and luminosity,X-Ray generators: for smaller, laboratory.The X-Rays pass through the crystals and the beam is then reflected in a space behind the crystal. The reflected pattern can be collected using regular photographic film and then analyzed by looking at the relative intensity of the spots.Using the goniometer, the diffraction pattern at differentangles is obtained. With this 3D pattern, the correct crystal structure is obtainedThe 3D structure obtained above is the electrondensity map of the crystal.

RESULTS AND DISCUSSION

Measurement of UV absorbance

We measured the absorbance spectra of the of the plumage colour of Nectarinia asiatica.There are basically three types of feather as mentioned previously in above paragraphs. The feathers are collected from the BITS Pilani campus area.A total of 18 feather were taken for the analysis of the measurement of UV present in the different areas of the sample.As generally we speculated that the more colourful and more vibrant feather posseses the heavier amount of UV in its different section.The feather are placed in order of their size in ascending order from smaller to larger ,yellow one being the most small and the black being the larger in a glass plate. Then the sample are then placed in the Gel doc system. The yellow feather possess more UV than the orange and black feather.It provides a clue why the vibrant colours are mostly used as the cues for the sexual mating in birds.

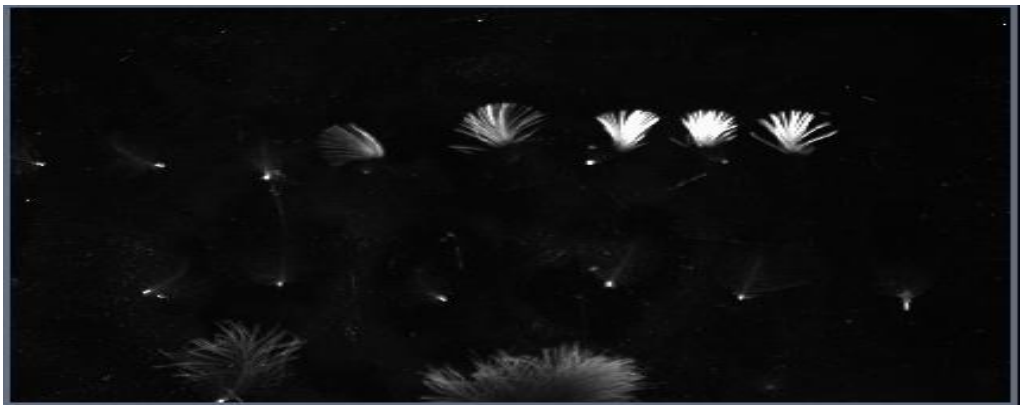

Fig 2;The most vibrant coloured pattern feather (yellow) possess more UV on the right Side top layer;the second layer contain UV only in traces in the calamus region(orange) Feather;the third layer being the most least amount of absorbance(black)feather.

Scanning Electron Micrograph Analysis

The feather sample which are placed in the SEM shows the characteristic feature which are described below. Different colors like blue black and yellow feathers are examined from various region say from the head ,breast and lesser coverts regions with different magnification .The structures shows a various heterogeneity all over the barb, barbules and rachis regions of the feather which we will be throwing light in the subsequent section.

Fig 3 (a)

Fig3 (b)

Fig 3 (c)

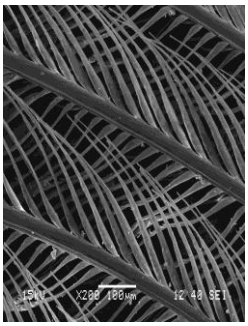

Fig 3(d)

Fig 3(e)

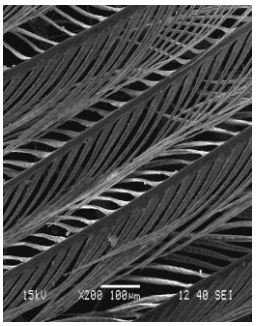

Fig 3(f)

Fig 3; Structural characterization of Nectarinia Asiatica feather. Scanned colour photographs [3(a)-3(b)-3(c)] shows the head region ,belly region and lesser coverts region.Scanning electron micrographs (SEM) panel [3(d)-3(e)-3(f)]shows the images of base region of blue , black and
yellow respectively.

The blue feather from the head region Fig 3(a) possess a beautiful symmetrical arrangement pattern
with the iridescent colors prominently being at the top region in a archaic manner and the hazy dull colour the base portion.The black feather of the bellyFig3(b) is almost dull and dark in itswhole topographical area .The calamus region feather is almost scattered being attached to the body.The yellow feather of the lesser coverts regionFig3(c)gives a radiant coloration partially glazing yellow surface with partially dark black colour.The Scanning electron

micrographs pictures Fig3(d) shows the base region of blue feather in a 100µm magnification with 200X zoom in a 15 k V.The rachis in the base region broadly arranged and slightly tapered while going towards the tip end of feather.The ramus are flat near the attachment of rachis goes on become like needle like structural. Using the ImageJ software we calculated the barb length averaging with 634.4 nm with standard deviation of 81.52.The average width of each barb being 26.25 nm with S.D of 10.24.The average length of rachis is 1094.03 nm and width around 42.42 having a S.D of 6.68.The black base region Fig 3(e) shows the rachis are arranged in a parallel manner .The barbs emerges out parallel in angle variation of about 108.5 nm with the next barb.Most barbs are conjoined with each other in a very nominal distance. The length of rachis averaging around 1356.24 nm and length of barb around 437.8 nm with an S.D of 131.52.In the Fig 3(f) the SEM analysis of the yellow feather shows the base region .The barbs bulges out in two different manner and there is lots of gaps among the barbs and the tapering end of barb overlap among each other.The average length of rachis is 1351.23 nm and average width is 17.78 nm with S.D of 4.86.The average length of barb is 321 nm with S.D of 48.45 and distance between each barb is like 36 nm with S.D of 7.96.

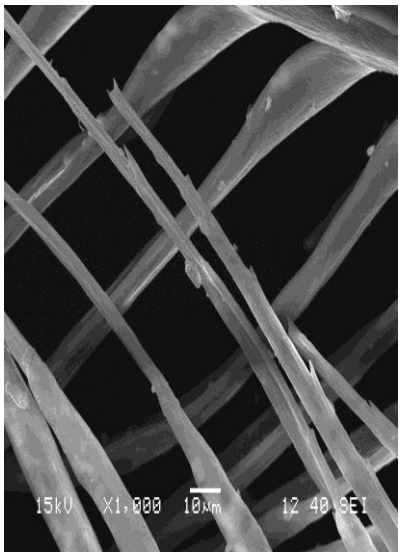

Fig 3 (g) blue base

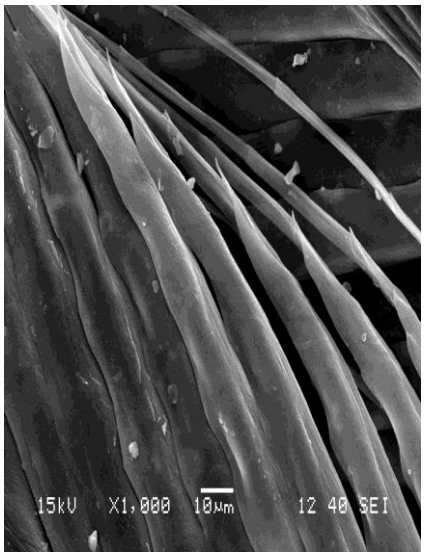

Fig 3 (h) black base

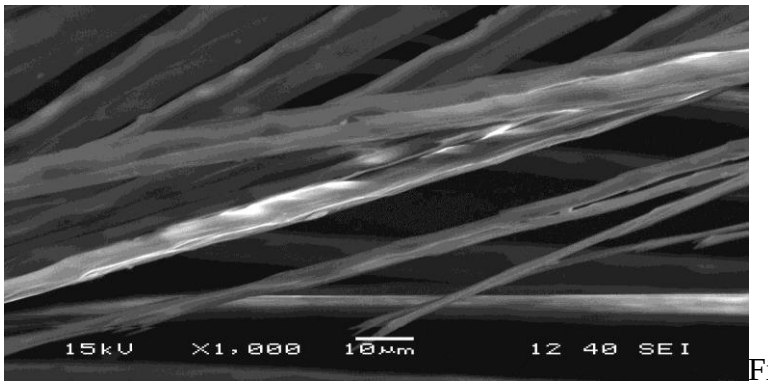

Fig 3 (i) yellow base

In the Fig 3(g) the barbules are stacked with each other showing the general pattern from being thick at the base and thinner towards the apex area. The average length of barbules is 1143.07 with S.D of 181.58 .The average width of barbules comes around 61.42 with S.D 33.65.In the Fig 3(h) the black base region of the feather show a wide variety of arrangement ,barbs placed one against another in a compact pack manner with average length of 1006.11 nm with S.D of 192.16.The thickness of the barb varies all along with an average of 119,34 nm and S.D of 101.83.The length of barbicles comes around 138.09 nm with S.D of 16.33.The yellow base feather are mostly arranged in parallel with the average barb length of 1342.43 nm with S.D of 22.94 Fig 3(i).The thickness of barb is substantially around 84.65 nm with S.D of 135.80.

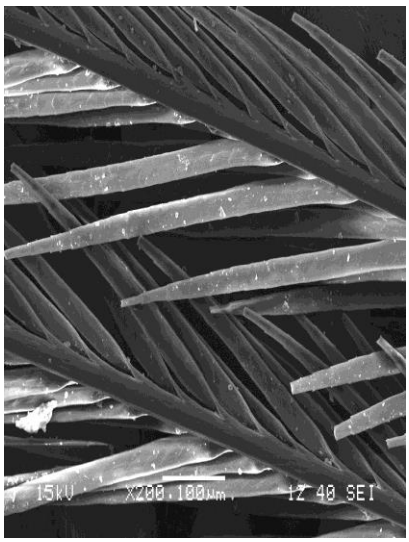

Fig 4 (a) blue tip [100µm,200X]

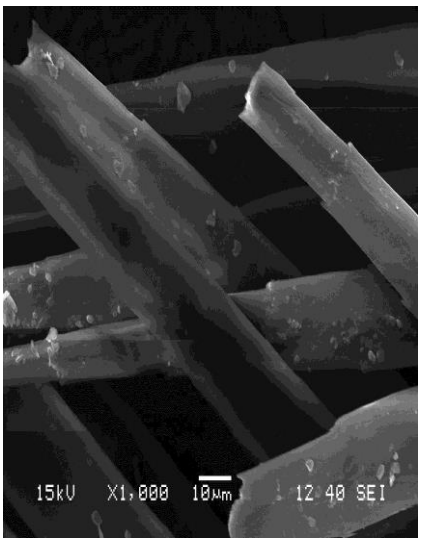

Fig 4(b) blue tip [10µm,1000X]

Fig 5(a) black tip [100µm,200X]

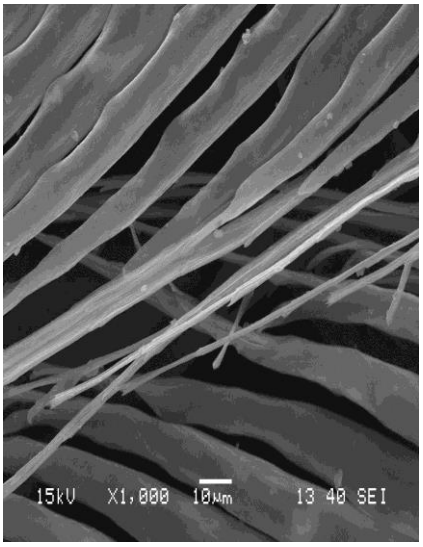

Fig 5(b) black tip [10µm 1000X]

Fig 6 (a) yellow tip [100µm,200X]

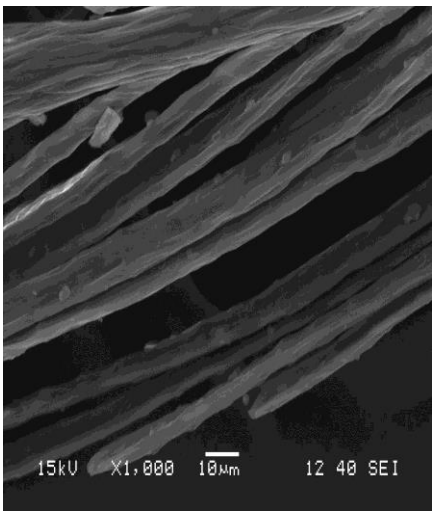

Fig 6(b)yellow tip[10µm,1000X]

We carried the structural pattern studies of the feather tip of the blue ,black and yellow.There is striking difference seen in the former base structure studies of the feather.It may be possible reason why the structural coloration differ in different region starting from the base being the dullest one while the tip and middle one carries the most beautiful and well crafted magnificient iridescent color. It is due to their difference in morphology in barbs and barbules which absorbs the different wavelength of light and reflecting it back producing a huge spectrum of array which makes the bird luminescent in appearance .The blue tip feather Fig 4 (a)in 100µm ,200X shows the cycas leaf like arrangement pattern. Some intertwined barb making a slight spiral like arrangement. Most of the them are cone shaped in structure and tip being little blunt end .The length and width of rachis are 1318.24 nm and 38.22 nm with a S.D of 3.69 and 5.89

respectively. The length and width of barb is 853.28 nm and 36.03 nm with S.D of 106.48 and 8.35 respectively. In Fig 4(b) in 10 µm 1000X we analysed the more magnified version of the tip end of the feather.It is cylindrical in structure ,the bigger cylinder engulping the smaller cylinder inside it .The barbules makes a criss cross arrangement with each other.The circular tip is little uneven in appearance 3/4th part being bulge out ¼ th part bulges in. The average length of barbules is 597.80 nm and width of base of the cylindrical region is about 138.13nm slightly wider than the apex region cyclinder which measures around 108.23 nm. In the Fig 5 (a)100µm, 200X the black tip feather is studied .The rachis are not in a parallel manner ,they are somewhat placed in semicircular way. The barbs on the both sides of rachis are differently arranged the top side being somewhat perpendicular in nature and the lower side are acutely arranged.The length of three visible rachis possess an average length of 839.52,1315.54,1290.06 nm. The average width of rachis 1,2, and 3 are 22.83,21.38,20.84 nm with a S.D of 2.30, 2.57 ,2.82 respectively. The average length of barb is 460 nm and width being 17.31 nm with an S.D of 6.06. Moving on towards the Fig 5(b) black tip 10 µm 1000X it shows a very different shape which other feather didn't show earlier .There is remarkable up and down appearance at the edge area of the tip seems like beads on strings enveloped by some kind of sheath .The average length of the tip of the barb is around 1382.72 nm .The different thickness at the tip region corresponding to zone 1 ,2, and 3 is around 86.72,43.13,17.54 nm respectively. In the fig 6 (a) 100µm 200X the yellow tip is put under investigation the barbs are thinner at the origin and thicker towards the ends. The rachis are scattered unevenly they are not arranged in a ordered way giving a scenario of algae in a water surface. The length of the rachis 1,2, and 3 is 1142.53 ,1252.92, 1262.93 nm with width averaging around 17.3, 15.8 and 16.46 nm respectively. The average length of barb is 822.97 nm with the short stem being 336.69 nm and long stem averaging at 430.46 nm. The width of the barb is 22.67 nm with S.D of 2.72. Fig 6 (b) 10 µ 1000Xyellow tip is seen like a several branch of rough , dull ,asymmetrical cylinder having a blunt end structure placed side by side. The length of the barb tip 1 and 2 is 1334.54 and 1092.652 nm respectively. The average width being 49.49 nm and 44.68 nm for the barb tip with an S.D of 12.17.

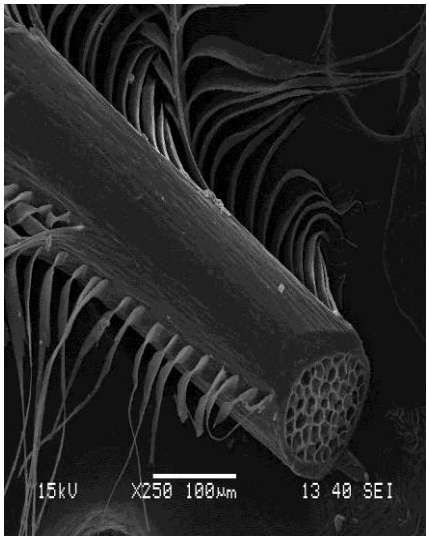

Fig 7 (a)Black feather tranverse section

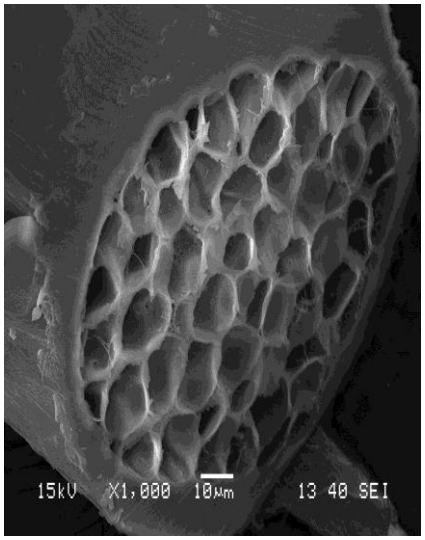

Fig 7(b) T S in 1000X

Fig 7 (c) T S in 5µm 5000X

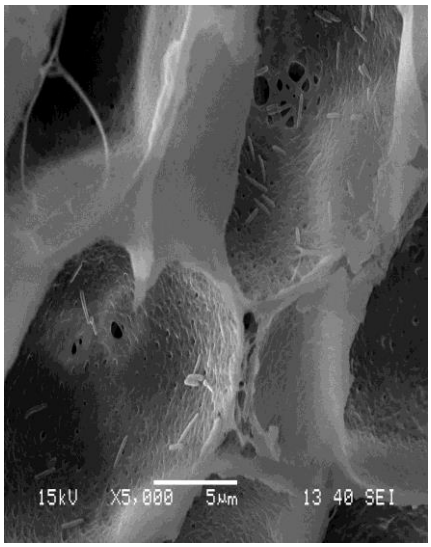

Fig 7(d) T S in 5 µm 5000X

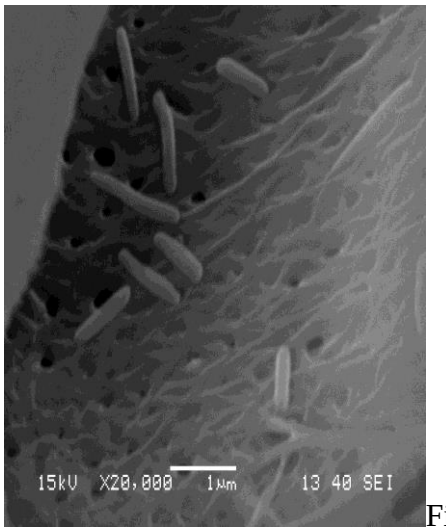

Fig 7 (e)T S in 1 µm 20000X

Fig 7;Scanning electron micrographs (SEMs) of tranverse section of black feather belly region.Fig 7(a) T S of belly feather ;Fig 7 (b) Magnified view of the cell ;Fig 7 (c) granular pigments are seen;

Fig 7 (d) closeup of a cell,small pigments granules are visible and recognizable;Fig 7 (e) the pigments granules are attached to the cell wall.

The black feather was cut transversely at the base region by a sharp blade . Then the tranverse section is washed with ethanol to preserve the structure from being damage and coated with the JEOL –JFC 1600(autofine coater).The coated sample are placed at the specimen stage of SEM for the characterization of the tranverse section of the black feather. The analysis of black tranverse section shows the following details.It is slightly round in structure with presence of several honeycomb like structure in the total surface which are joined together with each other Fig 7 (a).The different sponge cell in the T S didn't have the same color .There is huge variation in its coloring pattern with lots of heterogeneity Fig 7(b).The average area of the whole tranverse section is 850.5 nm with S.D of 73.47 and area of the small honeycomb like structure is 115 nm having at SD of 16.45.With simple shift in wavelength scale there is little magnification the presence of different colored granules are seen in the vicinity scattered in dense. These are specifically the melanin pigment which renders the black coloration of the feather Fig 7 (c).The rough uneven hollow cell possess the different granules. Each cell area averages around 517 nm with a SD of 128.44.The gap between each cell is about 57.8 nm.The pigment granules are of size 50 nm with SD of 11.49.The granular pigments are recognizable here Fig 7 (d).The pigment granules are seen most prominently in this magnification with each cell consist a average area of 637.51 nm and SD of 48.10.The standard average length of the granules is 54.91 nm. The pigment granules are attached to the cell wall Fig 7 (e) The average length of melanin pigment is about 178.01 nm with SD of 45.19 and average width of granules being aroud 28.57 nm with SD of 7.41

PIGMENT EXTRACTION RESULT AND DISCUSSION

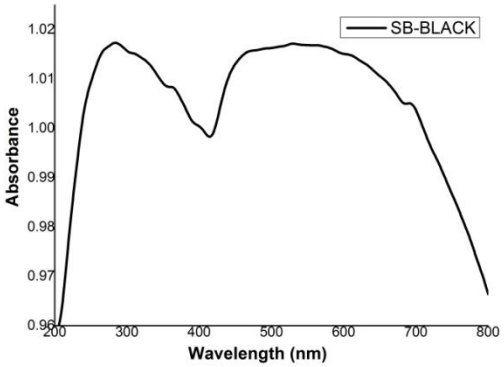

Fig 8 (a) Absorbance of Black feather

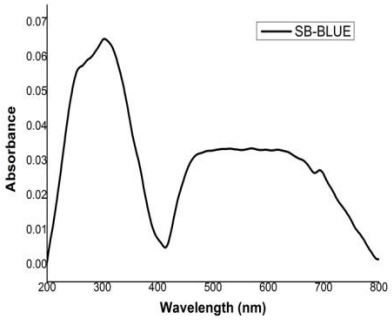

Fig 8 (b)Absorbance of blue feather

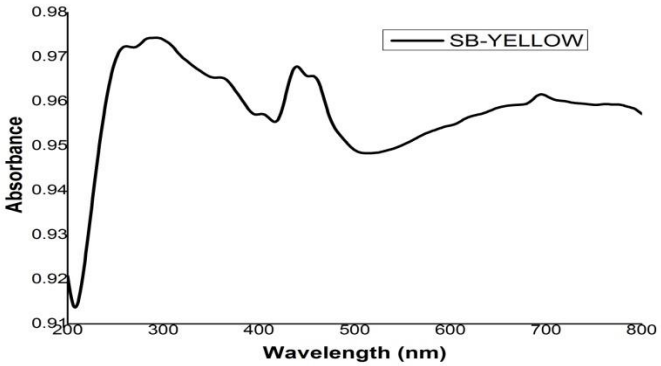

Fig 8 (c)Absorbance of Yellow feather

Melanin is strong absorbing pigment. Optical structures of melanin can now be incorporated into any model of structural coloration, substantially improving the link between the nanostructures and optical properties of biological materials. Melanin absorption increases strongly with decreasing wavelength.

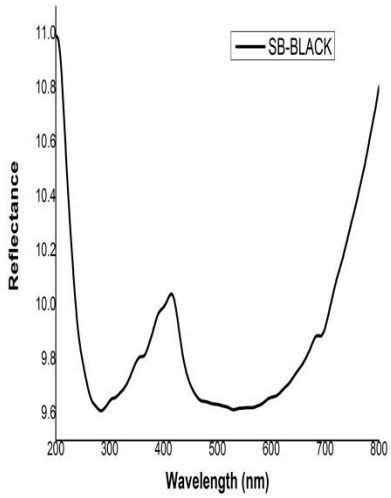

Fig 9(a)Reflectance of Black feather

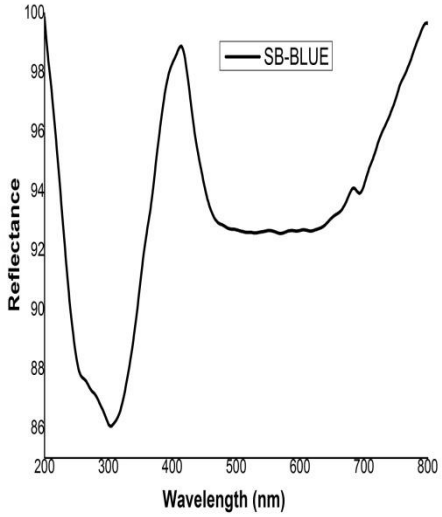

Fig 9(b) Reflectance of blue feather

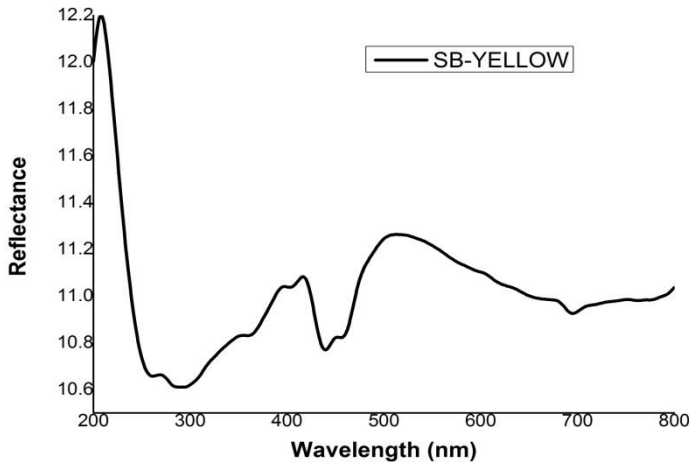

Fig 9 (c) Reflectance of Yellow feather

Reflectance spectra of the barbules Fig 9 (a-b-c) in the wavelength ranges from 200 -800 nm (long wavelength range) shows the characteristics of thin film interference. In other words the barbules behaves as part as thin film reflector. Yet the barbules internal structure with the highly ordered melanin layers that have a strong wavelength- dependent absorption , must contribute to the reflectance. For wavelength other than 500nm the modulations are similar but with the increasing wavelength the modulation slightly decreases due to dispersion.

Barbules as Multilayer and thin film

The barbules acts as a multilayer interference reflector because of refractive index contrast between the melanin and keratin layer. Generally ,when a multilayer comprises layers with

alternating thickness d_1 and d_h and refractive indices n_1 and n_h the reflectance peak wavelength for normal illumination in $\lambda_{max} = 2(n_1d_1 + n_hd$

X –RAY DIFFRACTION ANALYSIS AND DISCUSSION

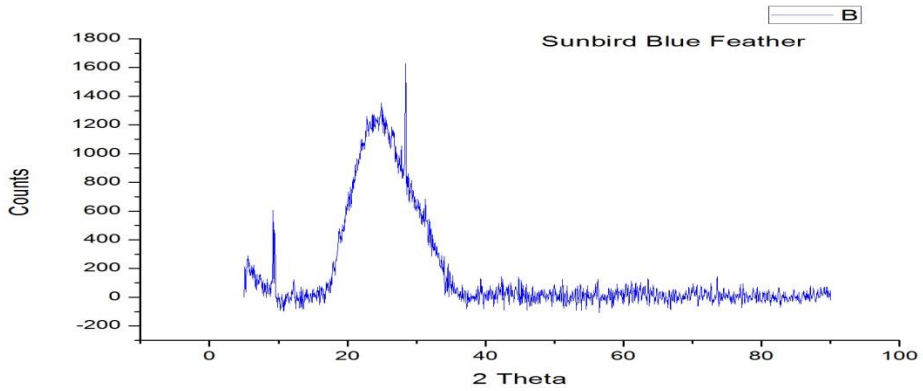

Fig 10 (a) XRD data for blue feather.

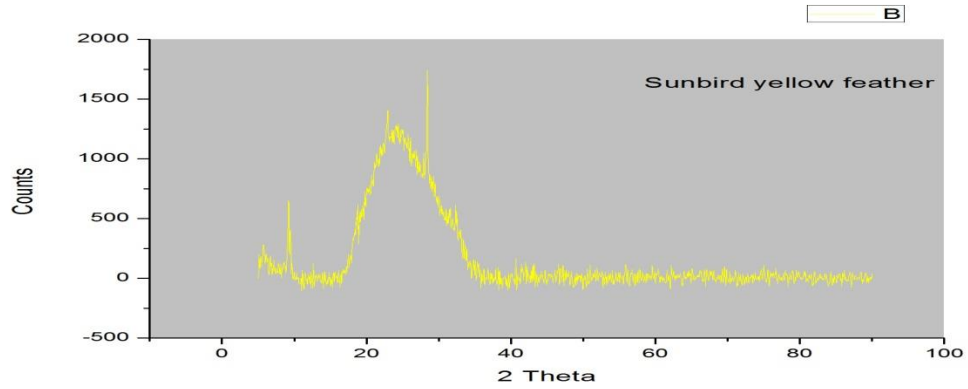

Fig 10 (b) XRD data for yellow feather.

We carried out the XRD data of the sunbird feather.There is presence of major peak and also some amounts of minor peaks in the data.The X axis contains the values of theta(θ) i. e. the detector position in accordance to the angle 2 theta(2θ).In a simple terms the detector moves in a circle round the sample.The Y axis contains the values of counts i. e. X ray intensity usually recorded as counts per second.The pattern shown in the graph Fig 10 (a) shows the metals like iron ,copper , zinc cobalt in a different amount.Some metals are more intense than the other.There is peak broadening in our data which indicate the presence of smaller crystallite size of nanocrystalline materials.Also there can be presence of inhomogenous composition in the sample and other background noises .The highest peak shown in the Fig 10(b) is of cobalt and nickel.

Quantitative analysis of the XRD data

We calculated the values of the unit cell dimension by correlating with the interatomic distances.Anything that changes the interatomic distances –temperature,substitutional doping,stress will be reflected by a change in the peak positions.We calculated the lattice parameters from the diffraction peak position by converting the observed peak position 2 theta(2θ) into d(hkl) values using Braggs law d (hkl) =λ/2sinθ

Milers indices are used for crytalographic direction .It is used to locate the origin .It is also used to identify at which the plane intercepts the x, y, z coordinates as fraction of unit length.If the plane passes through the origin,the origin of the coordinate system must be moved.The reciprocals of these intercepts are taken.

The Millers indices (hkl) of iron ,copper, zinc, are (100) (110) (000) respectively.

Iron is bcc ,copper is fcc and zinc and cobalt being hcp. The lattice constant of iron is 0.287 nm,while copper had l.c of 0.361 nm, zinc with 0.2665 nm, cobalt having the values with 0.2507.The atomic radius of iron , copper, zinc and cobalt are 0.124,0.128, 0.133, 0.125 nm respectively Fig 10 (a).

The metals found in the yellow feather are specifically cobalt and nickel. Cobalt has the hexagonal close packing arrangement with lattice constant of 0.2507 nm and atomic radius being 0.125 nm. Nickel possess an face centered cube with lattice constant of 0.352nm and atomic radius with 0.125 nm.

CONCLUSION

The bright colours of the purple sunbird *N. asiatica* are created by two types of feather barb: one filled with pigment granules and the other with quasi-ordered channel-type keratinous sponges nanostructures.

REFERENCE

1. Hausmann F et al.2002 Ultraviolet signals in birds are special

2. Oscar Vedder, Elske Schut, Michael J. L. Magrathand Jan Komdeur Functional Ecology 2010, 24, 417–425 Ultraviolet crown colouration affects contest outcomes among male blue tits, but only in the absence of prior encounters.

3. Richard O. Prum and Rodolfo H. Torres INTEGR. COMP. BIOL., 43:591–602 (2003) A fourier Tool for the Analysis of Coherent light Scattering by Bio –Optical nanostructures.

4. O. Prum et al. The Journal of Experimental Biology 202, 3507–3522 (1999) Coherent light Scattering by nanostructured collagen arrays in the caruncles of the Malgasy asities (Eurylaimidae:Aves)

5. Siefferman and Hill E.G *Evolution*, 59(8), 2005, pp. 1819–1828 evidence for sexual selection on Structural Plumage Coloration in Female Easttern Bluebirds (Sialis sialis)

6 N. R K rishnaswamy and C.R Sundersan Fascinating Organic Molecules from Nature

7. Stéphanie M. Doucet et al The Journal of Experimental Biology 209, 380-390 Iridescent plumage in Satin bowerbirds :structure mechanism and nanostructural predictors of individual variation in colours.

8. Alexandre Roulin, Anne-Lyse Ducrest Seminars in Cells and Developmental biology 24(2013)594- 608 Genetics of colouration in birds

9. Doekele G. Stavenga et al. Wilts The Journal of Experimental Biology 214, 3960-3967 Kingfisher feathers – colouration by feathers spony nanostructures and thin film.

10. Jose M.Medina,Jose et. al Classification of peacock feather reflectance using component analysis similarty factors from multi spectral imaging data.

11. Kevin J. McGraw et.al The Journal of Experimental Biology 205, 3747–3755 (2002) Different colour reveal different information ; how nutritional stress affects the expression of melanin –and structure based ornamental plumage .

12. Chelsea M. Berns The Evolution of Sexual Dimorphism.Understanding Mechanism of Sexual Shape Difference.

13. D'Alba et.al The Journal of Experimental Biology 215, 1272-1277 Relative contributions of pigments and biophotonic nanostructures to natural color production: a case study in budgerigar (Melopsittacus undulatus) feather.